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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/566,792	06/02/2006	Stuart Wilson	B019270063US00	7446

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EXAMINER	
BAUGHMAN, MOLLY E	
ART UNIT	PAPER NUMBER
1637	
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07/09/2007	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/566,792	WILSON ET AL.	
	Examiner	Art Unit	
	Molly E. Baughman	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *10 May 2007*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1-58* is/are pending in the application.

4a) Of the above claim(s) *35-58* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-34* is/are rejected.

7)☒ Claim(s) *33* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *31 January 2006* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *4/19/2007*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

1. Applicant's election without traverse of Group I, claims 1-34, in the reply filed on 5/10/2007 is acknowledged.

2. Claims 35-58 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 5/10/2007.

Claim Objections

3. Claim 33 is objected to because of the following informalities: the word, "staphyloccocus" is misspelled and should read, "staphylococcus." Appropriate correction is required.

Claim Rejections - 35 USC § 112

4. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claims 8-10, 20-21 and 26 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a. Claims 8-10 are confusing because claim 8 recites the limitation "the nuclease used to distinguish between nucleic acid molecules whose sensitivity in a subsequent process has been changed by enzyme acitivity in the sample and those whose sensitivity has not by digesting unchanged nucleic acid molecules"

in claim 6. There is insufficient antecedent basis for this limitation in the claim. For example, claim 6 only refers to "the nucleic acid molecule that is detected" and does not refer to nucleic acid molecules, nor any step of distinguishing. Clarification is required.

b. Claim 20 is confusing because it cannot be determined what is encompassed by "adding lambda exonuclease and mung bean endonuclease or exonuclease I." It is unclear which enzymes are required in the phrase, i.e. (1) lambda exonuclease and mung bean endonuclease, or (2) lambda exonuclease and exonuclease I, or (3) only exonuclease I.

c. Claim 21 is confusing because it cannot be determined what is encompassed by "at a concentration low enough to substantially prevent dsDNA digestion activity." It is unclear what such a concentration is and the specification does not provide further clarification.

d. Claim 26 is confusing because of the use of the trademark, "Taqman®." The claim scope is uncertain since the trademark or trade name cannot be used properly to identify any particular material or product (see MPEP 2173.05(u)).

Claim Rejections - 35 USC § 102

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

7. Claims 1-4, 6, 16-19 and 31 are rejected under 35 U.S.C. 102(b) as being anticipated by Sullivan et al. (US 4,720,458).

Sullivan et al. teach a method of detecting an enzyme in a sample comprising subjecting pBR322 DNA to restriction digestion, removing the terminal phosphates via a bacterial alkaline phosphatase (DK-47 or *E.Coli* APase), phosphorylating the reaction mixtures with labeled ATP and polynucleotide kinase, and subsequently determining phosphatase activity via gel electrophoresis (col.6, Radioactive End-Labeling Using HK-47 APase).

8. Claims 1, 6-8, 11-14, 17, 19, and 31 are rejected under 35 U.S.C. 102(b) as being anticipated by Stevens et al., "5' - Exoribonuclease 1: Xrn:1," Methods in Enzymology, 2001, Vol.342, pp.251-259.

Stevens et al. teach a method of determining enzyme activity of the exoribonuclease, Xrn1. Their method comprises subjecting various samples to Xrn1 and determining enzyme activity, wherein samples consisted of: ribosomal RNA comprising 5' labeled phosphate groups, labeling 3' poly(A) tail, labeled single stranded and double-stranded transcription plasmids digested with restriction enzymes. They noted that restriction digested DNA is hydrolyzed poorly (i.e. protected from nuclease digestion).

9. Claims 1, 2, 5, 14, 16, and 31-33 are rejected under 35 U.S.C. 102(b) as being anticipated by Mustonen et al., "Measurement by ^{32}P-postlabelling of 7-methylguanine levels in white blood cell Dan of healthy individuals and cancer patients treated with dacarbazine and procarbazine, "Carcinogenesis, 1991, Vol.12, No.8, pp.1423-1431.

Mustonen et al. teach a method of detecting enzymatic activity wherein various nucleic acid samples are treated with DNase II, MN (from Staphylococcus aureus), nuclease S1 (from Aspergillus oryzae), PAP (prostatic acid phosphatase from human semen), and/or SVPD (snake venom phosphodiesterase), and subsequently phosphorylated using radiolabeled ATP and T4 polynucleotide kinase, in order to detect the resistance of the DNAs to the enzymes tested (Materials and Methods, Dinucleotide Analysis, pp.1424). Phosphorylated terminal nucleotides of the DNA indicated successful digestion by the enzyme(s), thereby confirming the activity of the enzyme (pp.1427, Enzymatic digestion of modified dinucleotides and ROM-DNA, Figure 9, and pp.1429, right column). Confirmation of enzymatic digestion by the particular enzyme, some of which are associated with various infectious agents (i.e. Staphylococcus and Aspergillus), not only confirmed the presence of the enzyme, but also inherently confirmed the presence of the infectious agent to which the enzyme was associated.

10. Claims 1, 6-9, 11-12, 14-16, and 31 are rejected under 35 U.S.C. 102(b) as being anticipated by Mitsis et al., "Characterization of the interaction of lambda exonuclease with the ends of DNA," Nucleic Acids Research, 1999, Vol.27, No.15, pp.3057-3063.

Regarding claims 1, 6-9, 14, and 16, Mitsis et al. teach a method of detecting Lambda exonuclease in a sample by treating various labeled nucleic acids with the

enzyme. In two examples, they demonstrate that 5' labeled oligonucleotides are protected from nuclease digestion (particularly DNase I and micrococcal nuclease) by first treating the oligonucleotides with a polynucleotide kinase and Lambda exonuclease (pp.3058).

Regarding claims 11-12, and 15, Mitsis discusses creating double-stranded, blunt-ended DNA using a series of 5' -phosphorylated 20mer primers (Exonuclease Assays, pg.3058).

Regarding claim 31, Mitsis discusses the method, in different embodiments, carried out in a single tube (i.e. DNase I Protection, MCNase protection and Exonuclease - the various reactions were incubated with lambda exonuclease in a single tube).

11. Claims 1, and 23-26 are rejected under 35 U.S.C. 102(b) as being anticipated by Christensen et al., "Reverse Transcriptase Activity and Particle Production in B Lymphoblastoid Cell Lines Established from Lympophocytes of Patients with Multiple Sclerosis," Aids Research and Human Retroviruses, 1999, Vol.15, pp.285-291.

Christensen et al. teach a method for detecting reverse transcriptase activity wherein a nucleic acid sample is allowed to interact with the reverse transcriptase, and the interaction of the enzyme is detected using amplification via Taqman technology utilizing an oligonucleotide probe labeled with two fluorescent dyes (pg.286-287, RT Assays).

12. Claims 1, 6-8, 11-14, 16-19, 23-24, and 31-32 are rejected under 35 U.S.C. 102(b) as being anticipated by Aparicio et al., "A Streptomyces glaucescens

endodeoxyribonuclease which shows a strong preference for CC dinucleotide," Eur.J.Biochem., 1992, Vol.205, pp.695-699.

Aparicio teaches several methods of detecting *Streptomyces glaucescens* endodeoxyribonuclease in a sample, wherein after incubation with the enzyme, the nucleic acids have altered sensitivity. Identification of enzymatic digestion by the particular enzyme, from *Streptomyces glaucescens*, not only confirmed the presence of the enzyme, but also inherently confirmed the presence of the infectious agent to which the enzyme was associated [i.e. claims 1, and 32].

In one reaction, they digested bacteriophage DNA using *S.glaucescens* endonuclease (referred to here on as "SGE"). Next the DNA was dephosphorylated with calf intestinal alkaline phosphatase and labeled at the 5' termini using T4 polynucleotide kinase and radiolabeled ATP. The phosphorylated DNA was then digested with pancreatic DNase I and subsequently with snake venom phosphodiesterase, wherein after separating the digested DNA via thin-layer chromatography, the DNA had been completely digested to 5' -mononucleotides (pg.696, "Analysis of the 5' -terminal nucleotide of endonuclease-generated fragments") [i.e. claims 1, 12, 13, 16-17, 31].

In a second reaction, they digest DNA with SGE and label the 3' termini using terminal transferase and radiolabeled ddATP. The 3' labeled terminal nucleotide was digested by micrococcal nuclease and calf spleen phosphodiesterase, separated and the digested nucleotides were analyzed ((pg.696, "Analysis of the 3'-terminal nucleotide") [i.e. claims 1, 12, 17, 31]

In a third reaction, fragments digested by *S. glaucescens* nuclease were dephosphorylated with calf intestinal alkaline phosphatase and then labeled at the 5' termini with T4 polynucleotide kinase and radiolabeled phosphate. Labeling reactions were analyzed via electrophoresis (pg.696, "Size of DNA fragments generated by *S. glaucescens* nuclease") [i.e. claims 1, 12, 14, 16-17, 31].

In a fourth reaction, they determine the production of DNA fragments with 5'-protruding single-stranded tails by (1) labeling them with Klenow polymerase and radiolabeled nucleotide or (2) labeling the 5' termini with polynucleotide kinase and single-strand specific endonucleases such as mung bean nuclease or nuclease S1, and detecting the released nucleotides. They also test for the presence of 3' single-stranded overhangs by treating fragments labeled at their 3' termini by 3' terminal transferase and radiolabeled ddATP with exonuclease III, and subsequently detecting the released nucleotides. Control reactions run in parallel used λ DNA cut with EcoRI and HindIII (for 5' over-hangs), λ DNA cut with PstI (for 3' overhangs), and λ DNA cut with HaeIII (for blunt ends) ((pg.696, "Detection of single-stranded tails) [claims 1, 6-8, 11, 12, 14, 16-17, 19, 31].

In a fifth reaction, identification of the cleavage site of SGE was carried out according to enzymatic sequencing reactions using M13mp18 single-stranded DNA with 5'-phosphorylated universal M13 sequencing primer. In a parallel reaction, they create comparable DNA fragments to a sequencing ladder by the creation of double-stranded substrates by amplifying phosphorylation using the same primer via polynucleotide kinase, annealing the primer to the template and extending. The created substrates

were then digested with *S.glaucescens* DNase and all samples were analyzed via electrophoresis (pg.696, "Identification of the cleavage site of the nuclease") [i.e. claims 1, 12, 14-18, 23-24].

In a sixth reaction, plasmid pBR322 was doubly digested with *Ssp*I and *Hind*III, and labeled at the restriction site with Klenow polymerase and radiolabeled phosphate after purification. The end labeled fragments and SGE were incubated and analyzed via electrophoresis (pg.696, "Analysis of nuclease-DNA complexes on low-ionic-strength gels") [i.e. claims 1, 12, 14, 17-18, 31].

DNase I protection assays were done in a seventh reaction, using an *Ssp*I-*Hind*III DNA fragment, from pBR322 end-labelled with Klenow polymerase and radiolabeled ddATP, and pre-incubated with a synthetic oligonucleotide and SGE at various amounts. DNase I was then added to the reaction mixture, incubated, and analyzed via electrophoresis (pg.696, "DNase I footprinting" and Figure 2). [i.e. claims 6-8, 11-14, 17-19, 31].

13. Claims 1-10, 16, 21, 23-25, 27-30, and 32-34 are rejected under 35 U.S.C. 102(e) as being anticipated by Zhou et al. (US 2005/0118665 A1, priority date 6/9/2003).

Regarding claims 1, and 27-30, Zhou et al. teach a method of detecting enzyme activity, wherein the enzyme is immobilized on the surface of a solid support and the substance of interest is within proximity for an enzymatic reaction (abstract). In some embodiments, substrates include DNA, RNA, protein (pg.6, [0058]; pg.8 [0088]), and reactions could also include a plurality of different enzymes (pg.7, [0080]; pg.8, [0086]).

The enzymes can be immobilized using an antigen-antibody binding pair, i.e. biotin-streptavidin, immunoglobulin domains-protein A or antigen, etc. (pg.13 [0129]; pg.14 [0139]; pg.51 [0285]). The enzymatic reaction is detected by the generation of a detectable signal, which could be an increase or a decrease of the signal (pg.17 [0185]). Some examples of a detectable group can be a labeled moiety, such as a labeled phosphate group, nucleotide, or oligonucleotide (pg.18 [0194]; pg.46 [0236]).

Regarding claims 2-10, 16, and 33, enzymes of interest include kinases, protease, phosphatase, polymerase, hydrolase, transferase, a RNAse, a DNAse, endonucleases, exonucleases, and more specific enzymes include alkaline phosphatase, acid phosphatase, exodeoxyribonuclease I, exodeoxyribonuclease (lambda-induced), *Aspergillus* nuclease S1 (pg.2, [0016]; pg.6 [0058]; pg.8 [0086]; Table 1, pg.32, 34-36).

Regarding claims 6-10, and 21, in one embodiment, the detecting step could also comprise detecting a decrease in the amount of enzymatic activity (i.e. the substance is protected from enzymatic activity, or digestion) (pg.2, [0017]). They also discuss varying the concentration in relation to the desired enzymatic activity (pg.15 [0165-0166]).

Regarding claims 23-25, in some embodiments, the altered sensitivity of the nucleic acid molecule is detected using amplification (e.g. by PCR) of oligonucleotide tags, which includes rolling circle amplification-detection methods, competitive PCR, semi-nested PCR, Quantitative PCR, and FRET (pg.18 [0186-0187]).

Regarding claims 4-5, and 32-34, the enzymes or proteins are derived from the same source or the same species, or from different species, e.g. human, yeast, mouse, rat, bacteria, and C. elegans (pg.8 [0086]; pg.9 [0093]). The enzymes could be associated with a particular disease, or from a cell device from a pathological tissue (pg.9 [0095-96]).

Claim Rejections - 35 USC § 103

14. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

15. Claim 22 is rejected under 35 U.S.C. 103(a) as being unpatentable over Sullivan et al. (US 4,720,458) as applied to claims 1-4, 6, and 16-19 above, and further in view of Mitsis et al. (1999).

The teachings of Sullivan et al. are discussed above. Although Sullivan et al. discuss incubating a nucleic acid molecule which comprises blunt-ended dsDNA phosphorylated at both 5' ends with a phosphatase to permit phosphatase activity, they discuss further incubating the DNA with a polynucleotide kinase instead of adding lambda exonuclease to analyze the activity of the phosphatase.

The teachings of Mitsis et al. are discussed above. Particularly, Mitsis discusses reactions where blunt-ended DNA is first 5' phosphorylated to provide a substrate for lambda exonuclease (pg. 3058, Exonuclease Assays).

One of ordinary skill in the art would have been motivated to modify the method of Sullivan et al. to use lambda exonuclease instead of polynucleotide kinase in order to determine phosphatase activity because Sullivan demonstrates the benefits of using an enzyme (i.e. polynucleotide kinase) to aid in the detection of dephosphorylation by phosphatase, and Mitsis et al. not only state and demonstrate that lambda exonuclease has a preference for 5' phosphorylated ends (pg.3057, Introduction, 3rd paragraph, and entire document), but they also demonstrate in reactions with blunt-ended DNA substrates prepared plus or minus a 5' phosphate, that a 10-fold decrease in activity can be detected in the absence of the 5'-phosphate (pg.3060, Activity assays of lambda exonuclease, 3rd paragraph). Therefore, the skilled artisan would have had a reasonable expectation of success in using lambda exonuclease instead of polynucleotide kinase as the enzyme which aids in the detection of dephosphorylated DNA, as Mitsis shows that lambda exonuclease is sensitive to the presence or absence of 5'-phosphate. It would have been *prima facie* obvious to one of ordinary skill in the art at the time of the invention to carry out the claimed methods and use the claimed lambda exonuclease therein.

Summary

16. No claims are free of the prior art.

17. Heroux et al. (US 6,312,896), Kroeker et al., "Gene-Sized Pieces Produced by Digestion of Linear Duplex DNA with Mung Bean Nuclease," Biochemistry, 1978, Vol.17, No.16, pp.3236-3243, and Sriprakash et al., "The Specificity of I Exonuclease," J.Biol.Chem., 1975, Vol.250, No.14, pp.5438-5445, are noted as references of interest.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Molly E. Baughman whose telephone number is 571-272-4434. The examiner can normally be reached on Monday-Friday 8-5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Molly E Baughman
Examiner
Art Unit 1637

MEB 7/3/07

KENNETH R. HORLICK, PH.D
PRIMARY EXAMINER

7/3/07